METHOD OF DIAGNOSING BONE METASTASIS
OF MALIGNANT TUMORS

TECHNICAL FIELD

This invention relates to a method of diagnosing bone
5   metastasis accompanying malignant tumors such as breast
cancer, prostatic cancer and lung cancer; the invention
also relates to a method of evaluating the therapeutic
effect of drugs used to treat these diseases.

BACKGROUND ART

10   Bone metastasis of cancer is conventionally diagnosed
by examining clinical symptoms of the patient or images
taken by simple radiography, bone scintigraphy, CT, MRI,
etc.   From a visual viewpoint, bone metastases are
classified as a dissolution type, a hardening type or a
15   mixed type depending on the balance between bone
dissolution and formation at the site of bone metastasis.
While image diagnoses are highly reliable and useful, they
are generally too expensive to be used for screening and
monitoring purposes.

20   With the recent advances in the study of bone
metabolism, various markers have been developed as indices
of bone metabolism.   Markers of bone formation and
resorption are separately listed in Table 1.   Attempts are
being made to diagnose bone metastases of cancers using
25   those bone metabolic markers (Koizumi, M. et al., Bone
Metabolic Markers in Bone Metastases, J. Cancer Res. and
Clin. Oncol., 121:541-548, 1995).

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Table 1

Markers of bone formation		
(1) Type I procollagen peptides	.....	proliferation
C-terminal propeptide (PICP)		
N-terminal propeptide (PINP)		
(2) Alkali phosphatases	.....	matrix formation
total alkali phosphatase (ALP)		
bone alkali phosphatase (BALP)		
(3) Osteocalcin (OC)	.....	mineralization
C-terminal fragments		
intermediate portions		
intact		

Markers of bone resorption

(1) pyridinium cross-links

    total urinary pyridinoline·deoxypyridinoline (HPLC method)

    free urinary deoxypyridinoline (fDPD)

(2) pyridinium crosslinked collagen peptide fragments

    serum C-terminal telopeptide (ICTP)

    urinary C-terminal telopeptide (CTx)

    urinary N-terminal telopeptide (NTx)

(3) Tartrate-resistant acid phosphatase (TRAP)

(4) Galactosyl hydroxylysine (GHYL)

(5) Hydroxyproline

(6) N-terminal osteocalcin

Most of the bone metabolic markers have as their rationale the measurement of metabolic products that are

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released into blood and urine in the process of formation
and absorption of type I collagen which accounts for 90% of
the bone matrix. To be more specific, type I procollagen
which is synthesized during bone formation releases C- and

5   N-terminal propeptides when it is converted to type I
collagen and these propeptides serve as markers of bone
formation. In the process of bone resorption, the type I
collagen in the bone matrix undergoes metabolism to be
released into blood and urine; the measured blood and urine

10 levels of the released type I collagen serve as markers of
bone resorption.

Bone formation is known to consist of three major
phases depending upon the stage of proliferation and
differentiation of osteoblasts; the first phase is where

15 oesteoblasts proliferate and the matrix forms, the second
phase is for matrix maturation and the third phase is for
calcification, and different markers are known to appear at
different phases (Stein, G.S. et al.: Relationship of Cell
Growth to the Regulation of Tissue-Specific Gene Expression

20 during Osteoblast Differentiation, FASEB J., 4:3111-3123,
1990).

In the phase of osteoblast proliferation and matrix
formation, type I collagen forms actively and C- and N-
terminal propeptides appear in the blood. In the phase of

25 matrix maturation, bone alkali phosphatase (BALP) is
generated actively, causing BALP to be secreted into the
blood. At the stage of calcification, osteocalcin (OC)
appears. Bone formation is accelerated in OC-deficient

mice, suggesting that OC works as a suppressant of bone
formation (Ducy, P. et al.: Increased Bone Formation in
Osteocalcin-Deficient Mice; Nature, 382:448-452, 1996).

In the box of "Markers of bone formation" in Table 1,
5   "(1) proliferation" corresponds to the phase of osteoblast
proliferation and matrix formation, "(2) matrix formation"
to the phase of matrix maturation, and "(3) mineralization"
to the phase of calcification.

While there are various markers of bone formation,
10   they frequently behave differently depending upon the state
of the disease and it is important to realize specifically
in which phase each marker appears.

There are also various markers of bone resorption and
as with the markers of bone formation, metabolic products
15   of type I collagen are currently drawing special attention.
In type I collagen, collagen of a triple-stranded structure
occurs crosslinked with pyridinoline and deoxypyridinoline,
so when it is destroyed upon bone resorption, pyridinoline
and deoxypyridinoline cross-links having various sizes of
20   N- and C-terminal amino acids attached thereto are released
into the blood.

The measurements of resorptive markers include
that of cross-links alone (urinary pyridinoline and
deoxypyridinoline that are measured as free entities), that
25   of cross-links including C-terminal amino acids (CTx and
ICTP), and that of cross-links including N-terminal amino
acids (NTx). For generalized details about bone metabolic
markers, see the review article by Calvo et al. (Calvo,

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M.S. et al., Molecular Basis and Clinical Application of
Biological Markers of Bone Turnover, Endocrine Rev.,
17:333-368, 1996).

In bone metastasis, markers of bone metabolism behave

5   somewhat differently than in metabolic bone diseases such
as osteoporosis. Among formative markers, increased PICP
and BALP are observed in bone metastasis of prostatic
cancer which is a typical example of bone hardening
metastases but there is no significant increase in the

10   level of osteocalcin which rises in osteoporosis and other
metabolic bone diseases. The mechanism behind these
phenomena is not presently known. In breast cancer which
involves bone metastasis of a mixed type, the levels of
formative markers increase but not as much as in prostatic

15   cancer. In lung cancer which involves many cases of bone
metastasis of a dissolution type, there are no significant
increases in the levels of formative markers.

Among resorptive markers, ICTP differs from the other
bone metabolic markers in that it does not change greatly

20   after menopause but it has been found to increase in bone
metastasis of cancer. From the viewpoint of detecting bone
metastasis, ICTP may be considered a good marker that is
insensitive to enhanced bone resorption in the post-
menopausal stage. The levels of resorptive markers

25   increase not only in bone metastatis of lung cancer which
is mostly of a dissolution type but also in bone metastasis
of breast cancer which is mostly of a mixed type, as well
as in bone metastasis of prostatic cancer which is of a

hardening type.

DISCLOSURE OF THE INVENTION

While the studies of bone metabolic markers have seen remarkable advances, comparisons of their advantages and limitations are presently far from being thorough and given many various markers of bone resorption and formation, one cannot tell for sure which markers are currently the best in diagnosis of bone metastasis.

In clinical diagnosis of bone metastasis, choice of a marker is entirely up to the discretion of each doctor and no technique has yet been established that allows for systematic monitoring of bone metastasis.

An object, therefore, of the present invention is to provide a tool capable of systematic monitoring of bone metastasis.

Under the circumstances, the present inventors conducted intensive studies with a view to developing a tool for systematic monitoring of bone metastasis and found that this object could be attained by combining a marker (formative marker) that reflects the activity of osteoblasts with a maker (resorptive marker) that reflects the action of osteoclasts. The present invention has been accomplished on the basis of this finding.

Thus, according to one aspect of the invention, there is provided a method of diagnosing bone metastasis of malignant tumors using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

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According to another aspect of the invention, there is provided a method of evaluating the therapeutic effect of drugs using a marker that reflects the activity of osteoblasts and a marker that reflects the action of

5   osteoclasts.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing how three different markers begin to appear as osteoblasts differentiate;

Fig. 2 is a graph showing the relationship between

10   the efficacy of chemotherapy on patients with prostatic cancer involving bone metastasis and each of three markers, PICP, BALP and osteocalcin;

Fig. 3 is a graph showing the changes in a crossover index, osteocalcin/BALP, for patients with breast cancer;

15   and

Fig. 4 is a graph showing the changes in the ICTP level during treatment of breast cancer.

BEST MODE FOR CARRYING OUT THE INVENTION

We now describe the findings which were the basis for

20   accomplishing the present invention.

(1) Markers of bone formation as produced from osteoblasts:

As Fig. 1 shows, the differentiation of osteoblasts involves shifts in marker expression from PICP and PINP (type I procollagen peptides) through BALP (bone alkali phophatase) to osteocalcin (Stein, G.S. et al., FASEB J.,

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4:3111-3123, 1990).

(2) Reactions of BALP and osteocalcin to chemotherapy in patients with prostatic cancer and breast cancer who were

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suffering from bone metastasis:

The levels of two formative markers, BALP and osteocalcin, differed with the state of bone metastasis, as demonstrated in the following two Examples.

5 Example 1

During the period from October 1994 to April 1996, the levels of formative markers were measured in 43 prostatic cancer patients with bone metastasis and 46 prostatic cancer patients without bone metastasis. Of the
10 46 prostatic cancer patients who apparently had no bone metastasis, 29 had received prostatectomy or radiation therapy and the remaining 17 were newly diagnosed patients who received prostatectomy or radiation therapy after bone scintigraphy and serum sampling. The patients without bone
15 metastasis were aged 69 on average (ranging from 47 to 85 years old). The progress of prostatic cancer in these patients was as follows: four patients at stage A, 14 at stage B, 19 at stage C, and 9 at stage D1. Of the 43 patients with bone metastasis, 9 were newly diagnosed and
20 received hormone therapy after bone scintigraphy and serum sampling. The remaining 34 patients had received positive treatments by hormone therapy and/or chemotherapy at various time intervals from the start of these treatments. The patients with bone metastasis were aged 69 on average
25 (ranging from 53 to 83 years old).

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until analysis.

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Serum BALP was measured by enzyme immunoassay with an
ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was
measured by immunoradiometric assay with a BGP-IRMA kit of
Mitsubishi Chemical Corp.

5   The results are shown in Fig. 2, in which Z value is
defined by (measured value - average for the patients with
bone metastasis)/(standard deviation of a patient without
bone metastasis). In Fig. 2, CR, flare, NC, IMP, new and
PD have the following respective meanings.

10   CR   : complete remission

     flare : flare-up [the treatment was effective but the bone
              metastasis appeared to have progressed on a bone
              scan (scintigraphic) image].

     NC   : no change (no change was observed).

15   IMP   : improvement (a sign of improvement was recognized).

     new   : diagnosed to have a new bone metastasis.

     PD   : progression of disease (the disease was found to
              have progressed).

     For each of these patient groups, BALP and
20   osteocalcin had the following Z values.

Z value of BALP

         CR   :   2.18

         flare :   3.40

         NC   :   8.23

25   IMP   :   2.39

         new   :   1.82

         PD   : 24.50

Z value of osteocalcin

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        CR    :  1.30

        flare :  -0.18

        NC    :  0.04

        IMP   :  1.25

5       new   :  0.08

        PD    :  0.05
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Using these values, a crossover index (Z
osteocalcin/Z BALP) was calculated for each patient group.

Crossover index

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10      CR    :  0.596

        flare :  -0.053

        NC    :  0.005

        IMP   :  0.523

        new   :  0.044

15      PD    :  0.002
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[handwritten: complete remission BALP low Z 8.596 osteoc hi Z]

[handwritten: prog. disease BALP hi Z 0.002 osteoc. lo Z]

As is clear from the above data, BALP had a low Z
value (2.18) in the CR group in which the treatments proved
effective whereas it had a high Z value (24.50) in the PD
group in which the disease worsened. On the other hand,
20 osteocalcin had a high Z value (1.30) in the CR group but
had a low Z value (0.05) in the PD group. The crossover
index was 0.596 in the CR group but 0.002 in the PD group,
with a marked difference being observed between the two
groups. It can hence be concluded that the crossover index
25 allows for both diagnosis of the progression of bone
metastasis and evaluation of drug efficacy in the treatment
of the disease.

Example 2

As in Example 1, the levels of formative markers (BALP and osteocalcin) were measured in a total of 850 patients with breast cancer, 644 of whom had bone metastasis and 206 having no bone metastasis. The patients

5   with bone metastasis received chemotherapy or endocrine therapy targeted to the site of bone metastasis; they were classified into six groups, CR, NC, IMP, new and PD, according to the therapeutic efficacy achieved and the Z values of BALP and osteocalcin were determined in each

10   group. On the basis of the measured Z values, a crossover index was calculated for each patient group.

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until

15   measurement. Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp. The results are shown below.

20   <u>Z value of BALP</u>

        CR   : 0.741

        NC   : 1.514

        IMP   : 0.735

        new   : 2.021

25   PD   : 5.041

<u>Z value of osteocalcin</u>

        CR   : 0.267

        NC   : 0.237

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IMP    :  0.039

new    : -0.167

PD     :  0.516
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Crossover index

5   CR   : 0.360

       NC   : 0.157

       IMP   : 0.053

       new   : -0.083

       PD   : 0.102

[handwritten annotation: crossover index / complete remission 0.360 / prog. disease 0.102]

10   Fig. 3 is a graph showing the changes in crossover

index as observed in the respective groups CR, NC, IMP, new

and PD. Obviously, the crossover index for the group CR in

which the treatments proved effective was 0.360 whereas the

value for the group PD in which the disease worsened was

15   0.102, with a marked difference being observed between the

two groups. It can hence be concluded that as in the case

of prostatic cancer, the crossover index allows for both

diagnosis of the progression of bone metastasis of breast

cancer and evaluation of drug efficacy in the treatment of

20   the disease.

       The results of Examples 1 and 2 showed that the

patients with bone metastasis in group CR who were

effectively treated by drugs had high crossover index

values whereas the patients with bone metastasis in group

25   PD who changed for the worse without any therapeutic effect

had low crossover index values. It was therefore supported

that a crossover index between two osteoblast markers was

extremely effective in evaluating the degree of amelioration

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of bone metastasis (therapeutic efficacy of drugs).

In patients with prostatic cancer, the crossover index value of group CR was close to that of group IMP and so was the crossover index value of group NC to that of group PD; these data reflect the therapeutic efficacy for bone metastasis of prostatic cancer which was predominantly attributable to bone formation. The result from the patients with breast cancer was somewhat different in that the crossover index value of group NC was close to that of group PD whereas the crossover index value of group CR was not close to that of group IMP. The difference would have originated because breast cancer presents an image of bone metastasis to which bone dissolution is a more significant predisposing factor than bone formation. Therefore, in order to ensure that the progress of bone metastasis (the degree of aggravation) is diagnosed accurately, not only formative markers but also resorptive markers would have to be measured.

In Examples 1 and 2, the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs were diagnosed by measuring the crossover index between osteocalcin which is a marker associated with the phase of calcification and BALP which is a marker associated with the phase of matrix maturation. The present inventors also verified that the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs could be diagnosed by measuring the crossover index between osteocalcin which is a marker

associated with the phase of calcification and PICP and PINP which are a marker associated with the phase of osteoblast proliferation and matrix formation. Needless to say, osteocalcin can be replaced by any other markers that

5 are associated with the phase of calcification, PICP or PINP can be replaced by any other markers that are associated with the phase of osteoblast proliferation and matrix formation, and BALP can be replaced by any other markers that are associated with the phase of matrix

10 maturation.

In the past, several markers of bone formation have been identified and their levels have been individually measured to show that different markers were produced at different times depending on the stage in differentiation

15 and maturation of osteoblasts. However, it has not been shown clearly which of the formative markers should be exclusively used as indices of bone metastasis to reflect the fact that "differentiation and maturation of osteoblasts are suppressed by bone metastasis of cancer".

20 It was entirely unexpected from the prior art that the progress of bone metastasis and the efficacy of its treatment by drugs could be evaluated by the above-defined crossover index.

The bone to which cancer has metastasized is broken

25 down by osteoclasts. While several markers are known to be capable of evaluating the bone resorption that accompanies bone destruction, the degree of bone metastasis (worsening of the disease) and the effect of therapy in suppressing

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bone destruction can be evaluated definitely by identifying

ICTP (type I collagen carboxy-terminal telopeptide) which

is a collagen metabolite having a comparatively high

molecular weight, validating ICTP as a reliable marker

5   of bone resorption (see, for example, The Bone, Vol. 10,

No. 3, pp. 111-118, 1996). Described below is an example

showing the degree of bone metastasis and the effect of

therapy in suppressing bone destruction.

<u>Example 3</u>: ICTP Level in Treatment of Breast Cancer

10   ICTP levels were measured in 23 patients with breast

cancer who had received chemotherapy of bone metastasis by

CAF regimen (C, cyclophosphamide; A, doxorubicin or

Adriamycin; F, fluorouracil). The control group consisted

of 9 patients with breast cancer who had no bone metastasis

15   and received a CAF regimen as adjuvant therapy.

     After obtaining informed consent from all patients,

bone metabolic markers indicative of bone formation and

resorption were measured. At the onset of CAF treatment

and up to its end, blood samples were taken when bone

20   scintigraphy was performed once a month and the sera were

separated. The separated sera were stored frozen at -40°C

until analysis. A formative marker BALP was measured by

enzyme immunoassay with an ALKPHASE-B kit of Metra

Biosystems. A resorptive marker ICTP was measured by

25   radioimmunoassay with Orion Diagnostica. The serum CA 15-3

was measured by immunoradiometric assay with Centocor. The

measured values were expressed in terms of the average and

SE (standard error). A test of significance was carried

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out by analysis of variance (ANOVA) according to the Bonferroni method.

The results are shown in Fig. 4, from which one can see that the ICTP values in the patients of group PD

5   increased significantly over the ICTP values in the patients of group PR (partial response) and NC. The ICTP values of the patients of the "flare" group were significantly lower than those of the patients of group PD and substantially the same as those of the groups NC and PR

10   without flare-up. The terms PD, flare and NC in Fig. 4 have the same meanings as in Example 1. PR means "partial therapeutic effect recognized".

No statistically significant difference was shown by the values of BALP and CA 15-3.

15   It is therefore concluded that by measuring the ICTP level, one can evaluate the degree of exacerbation of cancer metastasis to bone.

According to the findings in Examples 1 - 3, the amelioration of bone metastasis (therapeutic effect) and

20   the degree of its exacerbation can be correctly diagnosed by monitoring two markers, one associated with osteoblasts and targeted to evaluation of therapeutic effect and the other associated with osteoclasts and targeted to evaluation of the worsening of the disease.

25   INDUSTRIAL APPLICABILITY

As described on the foregoing pages, the present invention provides a tool by which bone metastases caused by malignant tumors such as breast cancer, prostatic cancer

and lung cancer and the therapeutic efficacy of drugs for the cancers causative of such metastases can be diagnosed much more accurately than by the prior art methods.